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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/70, C07H 15/12, 17/00 C12N 15/00 — A1

(11) International Publication Number: WO 93/14768

(43) International Publication Date: 5 August 1993 (05.08.93)

(21) International Application Number: PCT/US93/00440

(22) International Filing Date: 27 January 1993 (27.01.93)

(30) Priority data:
826,070 27 January 1992 (27.01.92) US

(60) Parent Application or Grant
(63) Related by Continuation
US 826,070 (CIP)
Filed on 27 January 1992 (27.01.92)

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(81) Designated States: AU, BB, BG, BR, CA, CZ, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).

Published
With international search report.

(54) Title: METHODS AND COMPOSITIONS FOR NEUTRALIZING INTRACELLULAR NUCLEIC ACID-BINDING PROTEIN BIOLOGICAL ACTIVITY IN A CELL, INCLUDING METHODS AND COMPOSITIONS USEFUL TO REGULATE GENE FUNCTION

(57) Abstract

A method for neutralizing an intracellular nucleic acid-binding protein's biological activity in a cell by exposing the protein to an intracellular concentration of a single stranded (ss)DNA or RNA, or a double stranded (ds)DNA or RNA possessing a recognition motif for the protein, sufficient to cause binding of the protein to the (ss)DNA, (ss)RNA, (ds)DNA or (ds)RNA and thereby block the intracellular protein's biological activity.

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Description

Methods and Compositions for Neutralizing Intracellular Nucleic Acid-Binding Protein Biological Activity In a Cell, Including Methods and Compositions Useful To Regulate Gene Function

Technical Field

The present invention relates to methods and compositions for neutralizing intracellular protein biological activity on a cell, including methods and compositions useful to control gene function.

Background Art

Classical genetics was concerned with determining how genes participated in the development of an animal or of a plant. Recently, molecular genetics has permitted notable progress in satisfying that aim, providing an understanding of the importance of RNA processing and gene and chromosome structures in eukaryotes. Molecular genetics is now turning its attention to the study of the molecular basis for gene control in eukaryotic cells, and in particular in animal cells.

Molecular genetics has now provided proof that the regulation of genes exists at both the level of transcription and after transcription. It has also shown that specific patterns of gene regulation are important determinants in cellular and organic developmental decisions. Molecular genetics has provided a voluminous amount of information about the DNA sites required for transcriptional regulation and about the regulatory proteins that bind to these sites.

Although gene control can be applied to a number of

different molecular steps, the term "differential transcription" as is used in the context of gene control probably most frequently refers to differential protein synthesis rates in eukaryotes, as it does in prokaryotes. Differential processing of RNA transcripts in the cell nucleus, differential stimulation of mRNA in the cytoplasm, and differential translation of mRNA into protein are also important in eukaryotic gene control. Furthermore, in a few cases, chromosomal segments are amplified or rearranged so as to effect gene expression. For a general review of the subject see "Molecular Cell Biology", 2ed., Darnell, Lodish and Baltimore (1990) generally, and particularly chapter 11 thereof.

To date, there have been attempts at gene control in light of the considerable therapeutic potential which would be afforded by a successful and practical application of the technique. Illustratively, Zamecnik et al (Proc. Natl. Acad. Sci. (USA) (1986) 83:4143-4146) report the use of single-stranded (ss)DNA oligomers, homologous to specific target mRNAs, in a culture medium where the (ss)DNA oligomer is taken up by the cells and inhibits translation of specific mRNAs thereby preventing synthesis of its cognate protein. This method has been referred to as the antisense method of gene control. Sullenger et al (Cell (1990) 63:601-608) report the use of a high titer retroviral vector which infects cells and overexpress intracellularly an RNA sequence of interest to titrate out a viral RNA protein.

More generally, the genetic alteration of cells that is mediated by gene transfer and leads to the inhibition of viral replication has been termed "intracellular immunization". (Baltimore, Nature (1988) 335:395-396.) Intracellular immunization is perceived as being achievable by stable introduction of DNA into cells whose continuous expression

results in the inhibition of an essential viral gene. Several strategies employing stable gene transfer to inhibit gene function have been described, including antisense RNA (see Weintraub et al, Trends Genet. (1985) 2:22-25; Green et al, Annu. Rev. Biochem. (1986), 55:569-597; van der Krol et al, Bio. Techniques (1988), 6:958-976), ribozymes (Haseloff et al, Nature (1988) 334:585-591; Cotten et al, EMBO J. (1989), 8:3861-3866; Sarver et al, Science (1990) 247:1222-1225) and dominant-negative mutants (Friedman et al, Nature (1988) 335:452-454; Malim et al, Cell (1989) 58:205-214; Trono et al, Cell (1989) 59:113-120).

The high level of expression of an inhibitor-encoding DNA template, whether synthesizing antisense RNA, ribozyme, or expressing a dominant-negative mutant gene product, is however necessary to achieve effective inhibition of the target gene. The current inability to express sufficient levels of antisense RNA in a cell stably transformed with an antisense gene is considered to be a major impediment to the development of effective gene inhibition protocols (see van der Krol et al, supra). It is likely equally important in the design of effective inhibition protocols based on ribozymes or dominant-negative mutants. The idiosyncratic behavior of RNA polymerase II-based transcription units *in vivo* (see, e.g., Stewart et al, Proc. Natl. Acad. Sci. (USA) (1982) 79:4098 4102; Williams et al, Proc. Natl. Acad. Sci. (USA) (1986) 83:2566-2570; Williams et al, Blood (1988) 71:1730-1743) also limits the use of gene inhibition protocols in the application of the intracellular immunization concept to the live animal or human patient.

In light of the inestimable potential, including therapeutic potential for gene control, there is an accordingly strongly felt need for methods and compositions useful in gene control, including both unblocking and blocking

gene function.

Disclosure of the Invention

Accordingly, it is an object of this invention to provide novel methods and compositions useful in gene control.

It is another object of this invention to provide novel methods and compositions useful to unblock or block gene function.

It is another object of this invention to provide novel methods and compositions useful for neutralizing one or more intracellular nucleic acid-binding proteins' biological activity in a cell, where the protein functions as an intracellular transcriptional factor or in the intracellular assembly, processing and/or transport of nucleic acids.

The inventors have now surprisingly discovered that the above-noted objects of the invention, and other objects which will become apparent from the description of the invention given hereinbelow, are satisfied by using single-stranded DNA or RNA ((ss)DNA or (ss)RNA) or double-stranded DNA or RNA ((ds)DNA or (ds)RNA) containing a recognition motif for the targetted nucleic acid-binding protein: an intracellular transcriptional factor nucleic acid-binding protein or an intracellular protein which functions in the intracellular assembly, processing and/or transport of nucleic acids. In accordance with the invention the biological activity of a particular intracellular protein is blocked (or squelched) by exposing the intracellular protein to an intracellular concentration of such a (ss)DNA, (ss)RNA, (ds)DNA and/or (ds)RNA sufficient to cause binding of an amount sufficient of the intracellular protein of interest to the (ss/ds)DNA or (ss/ds)RNA to thereby block the biological activity of the

protein in the cell.

Best Mode for Carrying Out the Invention

The present invention is both conceptually and methodologically fundamentally different from other attempts at gene control or at unblocking or blocking gene function, and it has very broad applications to yeast, bacteria, plants, and animals. Instead of being based on the use of a (ss)DNA oligomer or of a (ss)RNA oligomer to intercept mRNA translation, it is based on the use of (ss)DNA, (ss)RNA, (ds)DNA or (ds)RNA to neutralize an intracellular nucleic acid-binding protein's, or proteins', biological activity. In accordance with the present invention cells are contacted with the (ss)DNA, (ss)RNA, (ds)DNA and/or (ds)RNA in a manner permitting the (ss/ds)DNA/RNA to be taken up by the cells to thereby obtain an intracellular concentration of (ss/ds)DNA or (ss/ds)RNA sufficiently high to inhibit the cellular function of the targeted protein(s) by binding to the protein(s) and thereby neutralizing it (them).

The ability of certain proteins to bind to either single-stranded or double-stranded nucleic acids is known. See, e.g., Krauss et al, Biochemistry (1981) 21:5346-5352 who disclose the binding of proteins from *E. coli* to oligonucleotides and the following review articles which pertain to DNA binding proteins, Gellert, "The Enzymes", Vol. XIIII (1981) 345-366; Wang, "The Enzymes", Vol. XIIII (1981) 332-343, Chase, Ann. Rev. Biochem. (1986) 55:103-136, and Kowalczykowski et al, "The Enzymes", Vol. XIII (1981) 374-444.

It is also known that gene control can occur at four separate levels: (1) transcription, which includes either initiation or termination; (2) nuclear processing of the primary transcripts; (3) cytoplasmic stabilization or

destabilization of RNAs; and (4) mRNA translation.

It is known that gene control occurs at each of these levels in eukaryote, but that not every gene either is or can be controlled at all four levels. And it is also known that the most widespread form of gene control in both eukaryotes and in prokaryotes occurs via regulation of transcriptional initiation. For a detailed discussion of the studies undertaken to date on gene control see Chapter 11 of Darnell et al, *supra*, which is summarized hereinbelow.

The regulatory sites in DNA and their cognate binding factors are known to be identifiable by molecular genetic techniques. To date, various studies have led to the recognition that many transcription factors are present in most if not all cell types, and that some of the widely distributed factors which are required for transcription of many genes are present in variant concentrations. These proteins are necessary for gene activity.

It is also known that most, and perhaps all, eukaryotic protein encoding genes require activators. The TATA box is generally located about 30 nucleotides upstream from the RNA start site of protein encoding genes. This start site is often called the cap site because primary RNA transcripts have a 5′ methylated cap. The proteins necessary for beginning transcriptions of protein encoding genes are known to be the TATA factor (transcription factor IID or $TF_{II}D$), RNA polymerase II, and accessory proteins ($TF_{II}B$ and $TF_{II}A$).

Initiation and transcription of eukaryotic genes by RNA polymerase II is accelerated by activators, which are intracellular proteins required for normal transcription of many, and perhaps all, eukaryotic genes. In yeast and other simple organisms, a single required upstream activating

sequence can be sufficient to obtain adequate initiation of transcription. This upstream activating sequence often interacts with a single protein factor which binds to a 15- to 20-bp segment in the sequence.

By contrast, mammalian genes are believed to contain multiple 15- to 20-bp protein-binding sites, with each site being necessary for maximal transcription. Vertebrate cells are known to encode many dozens, and perhaps hundreds, of such factors. In vertebrate cells, these factors vary in concentration in different cells. The presence and concentrations of these factors is crucial in the normal or abnormal development and differentiation of a cell.

It is known that eukaryotic DNA-binding proteins exhibit a limited number of structural designs. Three general structural designs have been found in DNA-binding proteins: the helix-turn-helix, the zinc-finger, and the amphipathic helix motifs. Helix-turn-helix proteins possess a remarkably conserved region encoding about 60 amino acids, called the homeobox, which is conserved well enough to be easily recognized in genes from frogs to mammals. These homeobox proteins are believed to contact specific DNA sequences. Zinc-finger proteins and amphipathic helical proteins, including leucine-zipper and helix-loop-helix proteins are also known to be regulatory proteins.

It is also known that at least eight different genes from invertebrates and from mammals contain both a homeobox and a second highly conserved region, termed the POU region, which is about 50 amino acids long and located about 50 amino acids away from the homeobox region, near the amino-terminal end of the proteins.

A high degree of conservation over these sequences and

span of evolution, indicating a fundamental role in gene activation, has been observed. Further, all of these DNA-binding protein are known to be able to increase transcription *in vitro* and *in vivo*.

The present invention targets at least one such intracellular nucleic acid-binding protein in a cell. One administers to this cell, or to a plurality of cells, including multicellular organisms, at least one (ss/ds)DNA or (ss/ds)RNA containing at least the recognition motif for the targeted nucleic acid-binding protein.

The intracellular nucleic acid binding proteins which are neutralized in accordance with the present invention illustratively include intracellular-nucleic acid binding proteins involved in the growth, the differentiation, the hormonal regulation, or the immune function of the cells being treated, or in the life cycle of a virus (e.g., HIV) associated with the cells or multicellular organism being treated, or in the assembly, processing and/or transport of nucleic acids in the cells or multicellular organism being treated. Illustrative genes whose expression can be advantageously controlled in accordance with the invention include nuclear transcription factors in general, e.g., proto-oncogenes such as c-myc, c-myb, fos, jun (Lewin, Cell (1991) 54:303).

The (ss/ds)DNA or (ss/ds)RNA is administered in an amount sufficient to obtain an intracellular concentration of (ss/ds)DNA or (ss/ds)RNA sufficient to bind an amount of the intracellular nucleic acid-binding protein sufficient to thereby neutralize (i.e., squelch) its function in the cell. Preferably the (ss/ds) DNA or (ss/ds) RNA is administered in an amount sufficient to obtain an intracellular concentration of about 10^{-8}M to about 10^{-4}M. At these concentrations the

(ss/ds) DNA or (ss/ds) RNA is capable of effectively neutralizing the nucleic acid-binding protein.

The methods and compositions of the present invention have broad applications for both unicellular and multicellular organisms, including, in particular, therapeutic potential for the latter. As noted above, the present invention can be applied to yeast and bacterial systems as well as to either plants or animals.

The invention is useful in any cellular system (yeast, bacterial, plant and animal) for the treatment of a variety of diseases, including viral infections, acquired or inherited hormonal, autoimmune, or malignant syndromes. It is also useful to block or unblock a particular gene function or modify a single or a plurality of characteristics of a cell or of an organism.

It may be used in yeast or bacterial systems to shut down a single or a plurality of disadvantageous or useless yeast or bacterial characteristic(s). For example it can be used in fermentation systems to eliminate a disadvantageous or otherwise unwanted yeast or bacterial gene product, e.g., a contaminant difficult to remove from a product being isolated from a yeast or bacterial fermentation or an intracellular component responsible for the degradation of the product being isolated, e.g., an undesired protease.

In plants and animals it can be used to imbue a plant or an animal with resistance to a particular pathogen, for enhancing an advantageous characteristic of the plant or animal, or for attenuating or eliminating a disadvantageous characteristic of the plant or animal.

In accordance with the invention, one identifies an

intracellular nucleic acid-binding protein of interest, e.g., a nucleic acid-binding protein involved with the genetic characteristic of interest. on the basis of the structure of the identified protein a (ss/ds) DNA or (ss/ds) RNA containing at least the recognition motif for the nucleic acid-binding protein is assembled, for example, using the known techniques of nucleic acid synthesis or cDNA sequence cloning. Once the (ss/ds) DNA or (ss/ds) RNA sequence of interest is identified, sufficient quantities of this material, in biologically pure form, may be obtained in accordance with known existing techniques. For example, sufficient quantities of this material may be synthesized, or the particular sequence of interest may be cloned, or the polymerase chain reaction (PCR) technique may be used.

The (ss/ds)DNA/RNA used in accordance with the present invention should contain at least a sequence, corresponding to the cognate binding site to the targeted nucleic acid-binding protein, which is typically in the range of being 15 to 50 nucleotides-long. It is also possible to use longer (ss/ds)DNA/RNA sequences in accordance with the present invention, particularly if elements of the sequence other than the cognate binding site sequence provide for improved intracellular stability, cellular penetration, etc.

In a preferred embodiment of the present invention (ds)DNA or (ds)RNA, and particularly (ds)DNA, is used to exploit the greater stability of these materials towards intracellular degradation. The inventors data have discovered that when the present nucleic acid-binding protein squelching method is practiced with (ds)DNA it is at least 200 fold more potent than the antisense oligomer approach. This appears to be due to the higher affinity between the protein (transcription factor) and its cognate DNA binding site, as compared to the interactions between the mRNA and the

antisense oligomer. Further, it also appears to reflect the much higher intracellular stability of (ds)DNA (and of (ds)RNA), as compared to the intracellular stability of the single stranded (ss)DNAs (and (ss)RNA) used in the antisense approach.

In accordance with the invention, the (ss)DNA, (ss)RNA, (ds)DNA and/or (ds)RNA can be administered to yeast, bacteria, including in vivo administration to plants and animals, by any known means of administration. For example, the compositions of the present invention may be administered to a patient, e.g., a human or an animal, in need thereof either systemically or locally. For example, the (ss/ds) DNA or (ss/ds) RNA may be administered in a liposomal formulation. In a preferred embodiment of the present invention, a liposomal formulation targeted to a specific tissue is used, for example, as is known, by associating the liposomal formulation containing the (ss/ds)DNA or RNA with an antibody specific to an antigen found on the surface of cells in the tissue of interest. Illustratively see U.S. Patent No. 4,948,590 to Hawrot et al and the references cited therein. Local administration of the compositions in accordance with the present invention may be carried-out by any known means, including the use of a catheter as described by Nabel et al in published PCT application no. W090/11734.

Methods for delivering oligonucleotides both into the cell and into the nucleus are available in the literature. For example, a review published in Synthecell Synthesis (1990) 2(1):1-66 discusses various chemical modifications which enhance the stability of oligonucleotides and the uptake of these into cells and cell nuclei. In particular, Vlahakos et al (J. Am. Soc. Nephrology (1992) 2(8): 1345-1354) describes a set of autoantibodies which penetrate cells and are deposited within nuclei of kidneys and other organs in vivo.

Oligonucleotides may be attached to these autoantibodies by any of several methods. For example, the method of Zuckermann et al (J. Am. Chem. Soc. (1988) 110: 1614 or Corey et al (Science (1987) 238: 1401) can be used to attach a 3'-S-thiopyridyl oligonucleotide and a cysteine residue in the autoantibody via a disulfide bond. Another method, involving linkage via a 2'-deoxyuracil-5-aminoheptyl unit with glutaraldehyde is given in Jablonski et al (Nucleic Acids Res. (1986) 14: 6115). Methods for delivering oligonucleotides which do not necessitate the modification of these oligonucleotides are also available: for example see Dreyfuss et al (Eukaryotic Nucl. (1990) 2:503-517). All of the above references are incorporated herewith by reference.

Having generally described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting.

1. Inhibition of human immunodeficiency virus-1 (HIV-1) replication:

HIV-1 encodes several regulatory genes (Cullen et al, Cell (1989) 58:423). One of these genes encodes a trans-activator, TAT, and greatly increases viral replication. TAT functions at least in part by increasing the level of transcription. The cis-acting sequences required for TAT trans-activation (termed TAR) are located downstream from the site of transcription initiation between +14 and +44 (Jakobovitz et al, MCB (1988) 8:2555; Garcia et al, EMBO J. (1989) 8:765; Selby et al, Genes Dev. (1989) 3:547). TAR sequences are present in all HIV MRNAs and can assume a stable stem-looped structure that is recognized by TAT (Muesing et al, Cell (1987) 48:691) and is required for TAT trans-activation *in vivo* (Berkhout et al, Cell (1989) 59:273). Thus

in this particular case, the TAR-RNA/TAT-protein complex constitutes the active trans-activator.

It has been shown that infection of cells with a high titer retrovirus vector, that contains HIV-1 TAR sequences, results in overexpression of TAR RNA, which leads to sequestering of TAT protein, thereby rendering the cells resistant to HIV replication. Therefore, application of appropriate concentrations of (ds)TAR oligonucleotides to the culture media of cells, resulting in uptake of TAR DNA by the cells, will render the cells resistant to HIV replication. The results of such an experiment will provide the basis to determine the range of TAR oligo-mediated inhibition of HIV replication and disease progression *in vivo*. *In vivo* application of TAR oligos may be accomplished via intravenous injection, where appropriate targeting/encapsulation aides (e.g., liposomes) may be used.

2. Inhibition of the Function of DNA Binding Proto-oncogenes: C-myc and other nuclear proto-oncogenes.

The nuclear proto-oncogene c-myc is expressed in almost all proliferating normal cell types, is down regulated in many cell types when they are induced to terminally differentiate, and abnormal expression has been associated with many naturally occurring neoplasms (Cole, Ann. Rev. Genet. (1986) 20:361; Kelly et al, Ann. Rev. Immunol. (1986) 4:317). The regulated and continued expression of c-myc has been shown to block terminal differentiation induced by physiological inducers (Hoffman-Liebermann et al, Mol. Cell. Biol. (1991) 11:2375).

The protein contains three structural domains that are homologous to domains found in characterized transcriptional factors: a leucine zipper (Landschulz et al, Science (1988)

240:1759); a helix-loop-helix motif (Murre et al, Cell (1989) 56:777); and an adjacent domain rich in basic amino acids (Davis et al, Cell (1990) 60:733. Sequence-specific DNA binding by the c-myc protein has been demonstrated (Blakwell et al, Science (1990) 250:1149; Prendergast et al, Science (1991) 251:186), and more recently c-myc has been shown to transactivate transcription of the a-prothymosin gene when c-myc protein is activated in quiescent rat fibroblasts (Eilers et al, EMBO J. (1991) 10:133).

Therefore, application of appropriate concentration of (ds)DNA containing c-myc specific binding motifs to various neoplasms will result in abolishment and/or reversal of their malignant phenotype. similar approaches would be applicable also to neoplasms involving apparent expression of other DNA binding proto-oncogenes (e.g., c-myb, fos, jun, etc.; Lewin, Cell, (1991) 54:303).

3. Experimental:

Krox24 is a zinc finger transcription factor that was identified as an immediate early response gene upon induction of cell growth and differentiation (for a review, see Bravo, "Growth Factor, Differentiation Factors, and Cytokines", pp. 324-343, Habenicht ed., Springer-Verlag, 1990). The inventors have observed that Krox24 antisense oligomers in culture mediums specifically block macrophage differentiation in myeloid leukemia cell lines as well as in normal primary myeloblasts, indicating that Krox24 plays as essential role in macrophage development. Under similar conditions, also a (ds)DNA oligomer containing the DNA binding site for Krox24 (Lemaire et al, Mol. Cell. Biol. (1990) 10:3456) was observed to inhibit TPA-induced microphage differentiation of human monoblastic leukemia HL60 cells. Oligomers of (ds)DNA were used at concentrations ranging from 1 μM to 80 μM. In some of

the experiments, inhibition of HL60-induced differentiation took place with (ds)oligomer concentrations as low as 1 μM, a concentration 100 fold lower than what is required to obtain inhibition with the antisense approach.

* * * * *

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

Claims

1. A method for neutralizing an intracellular nucleic acid-binding protein's biological activity in a cell, comprising exposing said protein to an intracellular concentration of at least one oligonucleotide, selected from the group consisting of single stranded DNAS, single stranded RNAs, double stranded DNAs and double stranded RNAs possessing a recognition motif for said protein, sufficient to block said protein's biological activity.

2. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the growth of said cell.

3. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the differentiation of said cell.

4. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the hormonal regulation of said cell.

5. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the immune function of said cell.

6. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the life cycle of a virus in said cell.

7. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the assembly of nucleic acids in said cell.

8. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the processing of nucleic acids in said cell.

9. The method of Claim 1, wherein said protein possesses the biological activity of being involved in the transport of nucleic acids in said cell.

10. The method of Claim 1, comprising using single stranded DNA.

11. The method of Claim 1, comprising using single stranded RNA.

12. The method of Claim 1, comprising using double stranded DNA.

13. The method of Claim 1, comprising using double stranded RNA.

14. The method of Claim 1, wherein said intracellular concentration is 10^{-8}M to 10^{-4}M.

15. The method of Claim 1, wherein said recognition motif is 20 to 50 nucleotides-long.

16. A method for neutralizing an intracellular nucleic acid-binding protein's biological activity in the cells of a multicellular organism, comprising exposing said protein to an intracellular concentration of at least one oligonucleotide selected from the group consisting of single stranded DNA, single stranded RNAs, double stranded DNAs, and double stranded RNAs possessing a recognition motif for said protein, sufficient to block said protein's biological activity.

17. The method of Claim 16, wherein said organism is an animal.

18. The method of Claim 16, wherein said organism is a plant.

19. The method of Claim 16, wherein said recognition motif is a nuclear transcription factor motif.

20. The method of Claim 16, wherein a single stranded or a double stranded TAR oligonucleotide is used.

21. The method of Claim 17, comprising administering said oligonucleotide to said organism as an encapsulated formulation.

22. The method of Claim 21, wherein said formulation is targeted.

23. The method of Claim 22, wherein said encapsulated formulation in a liposomal formulation.

24. The method of Claim 16, comprising administering said oligonucleotide systemically to said multicellular organism.

25. The method of Claim 16, comprising administering said oligonucleotide locally to said multicellular organism.

26. A biologically pure double stranded DNA molecule comprising a recognition motif for an intracellular nucleic acid-binding protein.

27. The DNA molecule of Claim 26, wherein said recognition motif is 20 to 50 nucleotides long.

28. The DNA molecule of Claim 26, wherein said recognition motif is a nuclear transcription factor motif.

29. The DNA molecule of Claim 28, wherein said recognition motif is the c-myc, c-myb, fos or jun motif.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00440

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/70; C07H 15/12, 17/00; C12N 15/00
US CL :435/172.3; 514/44; 536/24.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/172.3; 514/44; 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings National Academy of Sciences, Volume 86, issued May 1989, G. Anfossi et al, "An Oligomer Complementary to c-myb-Encoded mRNA Inhibits Proliferation of Human Myeloid Leukemia Cell Lines", pages 3379-3383, especially page 3381, Figure 3.	1-29
X	Journal of Immunology, Volume 140, No. 7, issued 01 April 1988, A. Harel-Bellan et al, "Specific Inhibition of c-myc Protein Biosynthesis Using An Antisense Synthetic Deoxy-Oligonucleotide in Human T Lymphocytes", pages 2431-2435, especially page 2434, Figures 3 and 4.	1-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search
21 April 1993

Date of mailing of the international search report
28 APR 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00440

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the National Academy of Sciences, Volume 88, issued May 1991, L. Han et al, "Inhibition of Moloney Murine Leukemia Virus-induced Leukemia in Transgenic Mice Expressing Antisense RNA Complementary to the Retroviral Packaging Sequences", pages 4313-4317, especially page 4316, Table 2.	1-29
X	Proceedings National Academy of Sciences, Volume 83, issued July 1986, J.T. Holt et al, "Inducible Production of c-fos Antisense RNA Inhibits 3T3 Cell Proliferation", pages 4794-4798, especially page 4797, figure 4.	1-29